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RESEARCH ARTICLE

Geographical pattern and ploidy levels of the weed *Solanum elaeagnifolium* (Solanaceae) from Argentina

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Abstract A total of 106 samples taken from natural Argentinean populations of the weedy *Solanum elaeagnifolium* (subgenus *Leptostemonum*) were studied cytologically to understand the impact of the different ploidy levels in its distribution and origin. Classical Feulgen staining was employed to determine mitotic chromosome numbers in all samples. 2C nuclear DNA content was determined by means of PI flow cytometry in eight samples of different ploidy levels. Principal component analysis and GIS tools were employed to compare altitude, annual precipitation and annual mean temperature among accessions. Three cytotypes were found: diploid (2n = 24) which is widespread, tetraploid (2n = 48) centered in western and southern Argentina, and hexaploid (2n = 72) which predominates in central Argentina extending as well to the east. The annual precipitation is significantly different between tetraploids and hexaploids. Cx-values ranged from 1.231 to 1.275 pg, with statistical differences (of about 24.5–50.9 Mbp, $p \leq 0.05$) among accessions. Diploids are the most widespread cytotype and have adapted to a number of very different habitats. Tetraploids live in arid or semi-arid regions with a mean annual rainfall less than 500 mm. Hexaploids are successful in colonizing wetter areas, where no tetraploids were found. Thus, the distribution of cytotypes may be associated with habitat differences, particularly soil moisture. The observed cytotype pattern and the differences in DNA content suggest multiple places of origin for the polyploidy of *S. elaeagnifolium* in Argentina.

Keywords Argentina · Chromosome number · Distribution · DNA content · Polyploidy · Soil moisture · *Solanum elaeagnifolium*

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Introduction

Solanaceae, with approximately 100 genera and ca. 2,500 species, is cosmopolitan and has its centre of diversification in South America (Hunziker 2001; Olmstead and Bohs 2007; Olmstead et al. 2008). It includes several species of great economic, ethnobotanic, scientific, and ornamental value, such as tobacco (*Nicotiana tabacum* L.), chili pepper (*Capsicum* spp.), tomato (*Solanum lycopersicum* L.), and potato (*S. tuberosum* L.). Other members of the family have been helpful to botanists in clarifying evolutionary processes, such as reproductive biology, chromosome

speciation or plant virology (Hunziker 2001). In addition, many groups within Solanaceae have been the subject of phylogenetic studies based on either molecular or morphological data (e.g. Olmstead and Bohs 2007; Olmstead et al. 2008).

The largest and most diverse genus of Solanaceae (and even one of the largest among angiosperms) is *Solanum* L., with approximately 1,100–1,400 species (Hunziker 2001; Bohs 2005). Almost one-third of its species belong to subgenus *Leptostemonum* (Dunal) Bitter—the so called 'spiny solanums'. This group includes food plants (e.g. eggplant, *S. melongena* L.; "naranjilla" or "lulo", *Solanum quitoense* Lam.), but also several weeds such as the tropical soda apple (*S. viarum* Dunal), the sticky nightshade (*S. sisymbriifolium* Lam.), the horsenettle (*S. carolinense* L.), and the buffalo bur (*S. rostratum* Dunal) (Levin et al. 2006).

The subject of this paper is one of these weeds: *S. elaeagnifolium* Cav. ("silverleaf nightshade" or "quillo"). It is a perennial herb up to 0.5–1 m high, covered with silvery white peltate hairs. It is highly morphologically variable through its distribution area, particularly in leaf shape and number of prickles (the 'spines' of the common name of the subgenus). Its reproductive strategies include propagation by means of seeds, creeping rhizomes, and root fragments (Fernández and Brevedan 1972). The species is adapted to semiarid regions with 300–600 mm annual rainfall and coarse textured, sandy soils (Boyd et al. 1984).

There is some question regarding the geographic origin, either North or South America, but Boyd et al. (1984) suggest the south-western United States and northern Mexico as the most likely centre of origin. Its range reaches Argentina, where is widely distributed, and where it has its southernmost limit in the Rio Negro province (Morton 1976). This species is a typical agronomic weed since it competes with crops and produces allelopathic compounds (Mkula 2006). Silverleaf nightshade has spread into many regions of the world beyond its native range, such as Australia (Cuthbertson 1976), Israel, Morocco, South Africa, Syria, Tunisia (Boukhris-Bouhachem et al. 2007; Mekki 2007) and Turkey (Ilçim and Behçet 2007). One of the attributes of *S. elaeagnifolium* is that it is a source of solasodine, a precursor of steroidal hormones (Maiti and Mathew 1967), and, as a result, there have been attempts at cultivation in Argentina (Trione and Cony 1988).

Cytologically, previous work on *S. elaeagnifolium* meiosis (Moscone 1992) demonstrated a euploid series (2x, 4x, and 6x) for populations growing spontaneously in Argentina, while in other regions of the world only diploids were found (Powell and Weedin 2005). This is an interesting fact because polyploidy is important for many aspects described in several review articles (e.g. Soltis and Soltis 1993; Soltis et al. 2003; Hegarty and Hiscock 2008; Leitch and Leitch 2008; Van de Peer et al. 2009). Autopolyploidy is a common phenomenon (Soltis and Soltis 1993; Ramsey and Schemske 1998), and the autopolyploid species might be successful for many reasons, such as intergenomic cross talk (Wendel 2000), positive changes in gene expression (Matzke and Matzke 1998; Soltis et al. 2003), alterations on plant/animal interactions (Thompson et al. 2004), or sexual dimorphism (Miller and Venable 2003). Polyploids often have a wider geographical range than their diploid parents (e.g. Whittemore and Olsen 2011), probably because they would be preadapted for habitats and resources off limits to their parents and not very adapted to the progenitor's habitat (Levin 2004).

The nuclear DNA content of *S. elaeagnifolium* and most members of *Solanum* subgen. *Leptostemonum* is mostly unknown, as to date, the DNA content of only ten species has been reported (Bennett and Leitch 2010). This is unfortunate because the nuclear DNA content of organisms (C-value for unreplicated haploid nuclei) is a valuable source of information (Bennett and Leitch 2005; Gregory 2005). Comparative C-values have helped understand genome size evolution (Bennett and Leitch 2005), and they are correlated with some features such as minimum generation time, life history, plant phenology, and important parameters for plant breeders, including frost resistance, biomass production, and ecological adaptations (Ohri 1998). Moreover, the nuclear DNA amounts are a useful tool in the study of phylogenetic relationships between taxonomically related groups (e.g., Ohri 1998; Zonneveld 2001).

The aims of this work were (1) To analyze *S. elaeagnifolium* cytotypes distribution in Argentina, (2) To determine the cDNA values of the different cytotypes, and (3) To discuss the relationships among cytotypes distribution, cDNA values, and environmental factors.

Materials and methods

Ploidy levels

Mitotic chromosomes were examined in squashes of root tips obtained from germinating seeds. Root tips were pretreated in saturated *p*-dichlorobenzene in water for 2 h at room temperature, fixed in 3:1 ethanol:acetic acid, and stained with alcoholic hydrochloric acid carmine or basic fuchsin. A total of 106 populations were determined for ploidy by this method (Table 1). Voucher specimens are deposited in the Herbarium CORD. In each sample, 5–30 individuals were analyzed. The number of individuals per sample is indicated in Table 1.

Estimations of DNA content

Ploidy levels were also estimated by flow cytometry in addition to root tip chromosome counting. Plants to be used for flow cytometry were grown from seeds collected in the field. Voucher materials are listed in Table 2. For every accession, 1–3 individuals were measured, three runs each. Nuclear suspensions were prepared according to Doležel et al. (2007): approximately 1 cm^2 of leaf tissue of *S. elaeagnifolium* and the appropriate piece of internal standard leaves were chopped with a razor blade in a glass Petri dish containing 1 mL of ice-cold Otto I solution (0.1 M citric acid and 0.5% v/v Tween 20). *Solanum lycopersicum* L. cv. Stupické (2C = 1.96 pg, Doležel et al. 1992) and *Zea mays* L. cv. CE-777 (2C = 5.43 pg, Lysák and Doležel 1998) were used as an internal reference standards (Table 2). The nuclear suspension was then filtered through a 45 µm nylon mesh into a 5 mL cytometry tube. Subsequently, 1 mL of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12\ H_2O$; Otto 1990), RNAse (50 µg/mL) and propidium iodide (50 µg/mL) were added to stain the DNA and to avoid staining of double stranded RNA. Samples were kept on ice and analyzed within a 10 min period in a FACSVantage flow cytometer (Becton–Dickinson, San José, California) using an Innova 300 laser at 488 nm and CELLQuest software (Becton–Dickinson, San José, California). The flow cytometer was calibrated with chicken red blood cells (CRBC), to optimize forward and side scatter and fluorescence parameters. Three DNA estimations were carried out for each plant (5,000–10,000 nuclei per analysis) on three different days to avoid errors due to instrumental drift. Nuclear DNA content was calculated as: (sample peak mean/standard peak mean) × 2C DNA content of the standard (in pg). Cx-values, representing the DNA content of one non-replicated monoploid genome with the chromosome number x (Greilhuber et al. 2005), were calculated as the 2C nuclear DNA amount divided by ploidy level.

The ploidy level was estimated in relation to the DNA peak of the check. A previously known diploid individual of *S. elaeagnifolium* was used as an internal standard. ANOVAs and Games Howell tests were employed to find differences in nuclear DNA among samples. For comparison purposes, *Solanum* DNA content in the genus were taken from the Plant DNA C-values Database (Bennett and Leitch 2010).

Data analysis

Spatial analyses were conducted by means of GIS tools: the software employed was DIVA-GIS (Hijmans et al. 2004), with data on altitude, annual precipitation and annual mean temperature obtained from the 2.5 spatial resolution WorldClim data base (Hijmans et al. 2005). These data were employed in ANOVA tests in order to detect bioclimatic preferences for each ploidy level. Longitude, latitude, altitude, annual precipitation and annual mean temperature were used as variables in a principal component analysis (PCA). All statistical studies were performed using SSPS for Windows, Version 10.0 (Small Waters Corporation, 1999).

Results

Ploidy levels and distribution

We identified three ploidy levels based on x = 12: diploid (Fig. 1a), tetraploid (Fig. 1b) and hexaploid (Fig. 1c). The geographic distribution of each is presented in Fig. 2a, b. In the center of the country, the diploid area overlaps with the tetraploid and the hexaploid areas, while hexaploids and tetraploids are allopatric, with only two populations coexisting in a small area (Fig. 2a, b). However, a distribution pattern can be detected. The diploid cytotype (2n = 24, found in 36% of the samples) had the widest distribution, extending all the way west to Chile. The tetraploid cytotype (2n = 48, in 32% of the samples) is especially

Table 1 List of the *Solanum elaeagnifolium* samples studied, their collector numbers, ploidy level and provenance

Collector	Provenance
2x	
NI 1466	ISRAEL
(9, 42)	
NI 1503	MEXICO. Durango State, Mapimi
(10, 10)	
NI 1451	CHILE. Copiapo Prov.
(18, 105)	
NI 1452	CHILE. Atacama Prov., Vallenar, Marañón
(15, 112)	
AAC, ANS 369	CHILE. Atacama Prov., Paipote
(19, 113)	
RS 3881	Catamarca Prov., Capayán Dept., Casa de Piedra
(14, 110)	
AAC, EM, FE, LS 977	Catamarca Prov., La Paz Dept.
(9, 81)	
AH 25228	Catamarca Prov., Capayán Dept.
(10, 49)	
NI 1536	San Juan Prov., Iglesia Dept.
(5, 10)	
NI 1262	San Juan Prov., Jáchal Dept., Huaco
(8, 65)	
NI 1468	San Juan Prov., Jáchal Dept., RN 40, km 243
(1, 10)	
NI 1254	San Juan Prov., Jáchal Dept., Ing. Matías Sánchez
(16, 84)	
NI 1529 #	San Juan Prov., Angaco Dept., RP 436, Quebrada Paso de las Burras
(10, 62)	
NI 1251	San Juan Prov., Albardón Dept., Baños La Laja
(16, 85)	
AAC, ANS, RP 1011	San Juan Prov., Ullún Dept.
(10, 42)	
AAC, ANS, RP 1014	San Juan Prov., Ullún Dept.
(7, 88)	
GB 1944 #	San Juan Prov., Caucete Dept.
NI 1448	San Juan Prov., Caucete Dept., RN 141, Bermejo
(5, 10)	
NI 1269	San Juan Prov., Caucete Dept., RN 141, km 117
(2, 19)	
JA, EM 1483	Mendoza Prov., Las Heras Dept., Picheuta
(15, 93)	
NI 1443	Mendoza Prov., Las Heras Dept., Uspallata, El Chacay
(17, 98)	
JA, EM 1480	Mendoza Prov., Las Heras Dept., Uspallata
(16, 105)	

Table 1 continued

Collector	Provenance
JA, EM 1481 (17, 93)	Mendoza Prov., Las Heras Dept., Uspallata
NI 1453 (9, 52)	Mendoza Prov., Lavalle Dept., Reserva prov. Telteca
JA 1418 (14, 95)	Mendoza Prov., Capital Dept.
NI 1240 (13, 78)	Mendoza Prov., Capital Dept.
FR *s.n.* (15, 137)	Mendoza Prov. Capital Dept.
EF, OB 1110 (11, 60)	San Luis Prov., Ayacucho Dept., RN 20, km 390, La Chañarienta
NI 1444 (10, 60)	Santiago del Estero Prov., La Banda Dept., El Salado, Est. Exp. INTA
AH, EM 24868 (10, 86)	Santiago del Estero Prov., Atamisqui Dept., Isla Verde
MS 3 (8, 50)	Córdoba Prov., Cruz del Eje Dept., RN 38
EM 191 (9, 45)	Córdoba Prov., Pocho Dept., Chancaní
EM, GB 34 (12, 67)	Córdoba Prov., Tulumba Dept.
LB, LG 522 (16, 104)	Córdoba Prov., Tulumba Dept., RN 60, km 900
EF 823 (9, 45)	Córdoba Prov., San Justo Dept.
EF 831 (7, 53)	Córdoba Prov., Rio Primero/San Justo Dept.
RJ 34 (6, 44)	Córdoba Prov., San Justo Dept., Mar Chiquita
GB 76 (10, 42)	Neuquén Prov., Confluencia Dept
LB, RJ, AS 503 (8, 50)	Formosa Prov., Patiño Dept., Las Lomitas
NI 1478 (9, 38)	Santa Fe Prov., 9 de Julio Dept., Logroño
EDF 799 (1, 25)	Santa Fe Prov., San Cristóbal Dept., RP 39
4x	
RS, AAC, GB 3589 (2, 48)	Salta Prov., Rosario de la Frontera Dept., Rosario de la Frontera
NI 1511 (8, 30)	Catamarca Prov., Tinogasta Dept., Fiambalá
NI 1512 (10, 49)	Catamarca Prov., Andalgalá Dept., Andalgalá, Hotel Provincial de Turismo

Table 1 continued

Collector	Provenance
NI 1469 (8, 40)	Catamarca Prov., Capital Dept., San Fernando del Valle de Catamarca
NI 1508 (9, 37)	Catamarca Prov., El Alto Dept., RP 11, km 2
NI 1263 (14, 135)	La Rioja Prov., Chilecito Dept., Guandacol
AH, JZ, EM 25403 (10, 48)	La Rioja Prov., General Peñaloza Dept., RN 38, km 1,070–1,071
NI 1212 (14, 92)	La Rioja Prov., Chamical Dept., Chamical
AH, JZ, EM 25396 (10, 55)	La Rioja Prov., General Belgrano Dept., RN 38, km 230–232
EF 79 #	La Rioja Prov., San Martín Dept, between Tello and Chepes
AH, JZ, EM 25395 (8, 37)	La Rioja Prov., General Belgrano Dept., RN 38, km 217–218
LG 232 (9, 19)	La Rioja Prov., Capital Dept., Bazán
LV, EM 850 (2, 15)	Mendoza Prov., Luján de Cuyo Dept.
NI 1435 (18, 142)	Mendoza Prov., Rivadavia Dept., Campamentos IFONA
JA, AC, EM 1472 (1, 20)	Mendoza Prov., San Carlos Dept., La Consulta
NI 1434 (14, 92)	Mendoza Prov., Santa Rosa Dept., Ñacuñán Reserve
NI 1436 (18, 130)	Mendoza Prov., Santa Rosa Dept., Ñacuñán Reserve
NI 1244 (4, 14)	Mendoza Prov., La Paz Dept.
NI 1245 (5, 15)	Mendoza Prov., General Alvear Dept.
NI 1538 (8, 33)	Mendoza Prov., San Rafael Dept., RN 143
NI 1276 (3, 16)	San Luis Prov., La Capital Dept., El Chorrillo
GB 2010 #	Córdoba Prov., Minas Dept., San Carlos Minas
AH, JZ, EM 25385 (4, 19)	Córdoba Prov., Cruz del Eje Dept., Villa de Soto
LB, EM 449 (50, 277)	Córdoba Prov., Tulumba Dept., Cerro Colorado
NI 1540 (9, 42)	La Pampa Prov., Chalileo Dept., RN 143
NI 1565 (2, 6)	La Pampa Prov., Capital Dept., Colonia Lagos

Table 1 continued

Collector	Provenance
NI 1541	La Pampa Prov., Limay Mahuida Dept., Limay Mahuida
(8, 40)	
LG 237	La Pampa Prov., Limay Mahuida Dept.
(5, 18)	
AAC, ANS 439	La Pampa Prov., Utracán Dept., Ataliva Roca
(9, 20)	
LG 249	La Pampa Prov., Limay Mahuida Dept.
(4, 41)	
LG 239 #	Neuquén Prov., Confluencia Dept., Neuquén
NI 1544	Río Negro Prov., Pichi Mahuida Dept., RN 22
(9, 52)	
NI 1545	Río Negro Prov., Pichi Mahuida Dept.
(2, 10)	
NI 1546	Buenos Aires Prov., Villariño Dept., RN 22
(5, 18)	
NI 1416	Buenos Aires Prov., Bahía Blanca Dept., Altos de Palihue
(15, 114)	
GB 2308	Buenos Aires Prov., Tornquist Dept., Sierra de La Ventana
(3, 14)	
GB 2334	Chubut Prov., Biedma Dept., Puerto Madryn
(2, 10)	
GB 2340	Chubut Prov., Rawson Dept.
(4, 15)	
6x	
LG, AAC, ANS, SV 214	Salta Prov., General Güemes Dept.
(3, 13)	
NI 1535	Catamarca Prov., Paclín Dept., Cuesta La Merced
(4, 10)	
RJ 37	San Luis Prov., Junín Dept., Merlo
(16, 132)	
RJ 39 #	San Luis Prov., Junín Dept., Merlo
(2, 16)	
AH, RJ *s.n.*	San Luis Prov., Junín Dept., Merlo
(2, 12)	
LG 24	San Luis Prov., La Capital Dept., San Luis
(2, 15)	
AAC 220	San Luis Prov., Junín Dept., RN 146
(21, 191)	
AH, EM 24869	Santiago del Estero Prov., Capital Dept., Sgo. del Estero
(2, 13)	
AH, LB, EM 25089	Santiago del Estero Prov., Ojo de Agua Dept., Villa Ojo de Agua
(9, 83)	
LB, LG 534	Córdoba Prov., Sobremonte Dept., San Francisco del Chañar
(1, 22)	

Table 1 continued

Collector	Provenance
AAC 437 (3, 12)	Córdoba Prov., Ischilín Dept., Masa
MA 12 (1, 8)	Córdoba Prov., Ischilín Dept., Deán Funes
L.M *s.n.* (9, 19)	Córdoba Prov., Punilla Dept., Cosquín
EM 161 (3, 30)	Córdoba Prov., Punilla Dept., Santa María de Punilla
EM 70 (2, 25)	Córdoba Prov., Capital Dept., Córdoba
EM 71 (12, 140)	Córdoba Prov., Capital Dept., Córdoba
CC 1 (2, 15)	Córdoba Prov., Capital Dept., Córdoba
RJ 35 (4, 38)	Córdoba Prov., San Javier Dept., RN 148
RJ 36 (8, 87)	Córdoba Prov., San Javier Dept., San Javier
EM 109 (2, 60)	Córdoba Prov., Calamuchita Dept., Falda del Sauce
EM 180 (13, 122)	Córdoba Prov., Calamuchita Dept., RP 5
LB, EM 449 (50, 277)	Córdoba Prov., Tulumba Dept., Cerro Colorado
EM, AH 245 (7, 63)	Córdoba Prov., San Justo Dept., Jeanmaire
EF 825 (2, 27)	Córdoba Prov., Rio Primero/San Justo Dept.
AL 17 # (6, 28)	Santa Fe Prov., Castellanos Dept., Rafaela
AH, GB, EM 24860 (1, 54)	Entre Ríos Prov., Paraná Dept., Toma Vieja
MS 1 (10, 30)	Buenos Aires Prov., Rojas Dept., Rafael Obligado

Unless indicated, all materials are from Argentina. *AAC* A. A. Cocucci, *AC* A. Chicarelli, *AH* A. T. Hunziker, *AL* A. M. Luchetti, *ANS* A. N. Sérsic, *AS* A. Schinini, *CA* M. C. Acosta, *CC* C. A. Chiale, *EF* E. Di Fulvio, *EF* Eva Filippa, *EM* E. A. Moscone, *FE* F. Ehrendorfer, *FR* F. Roig, *GB* G. E. Barboza, *JA* J. A. Ambrosetti, *JZ* J. A. Zigaldo, *LB* L. M. Bernardello, *LG* L. Galetto, *LS* L. Schratt, *LV* L. Del Vitto, *MA* María C. Acosta, *MS* M. A. Scaldaferro, *NI* introduction number of IADIZA (Mendoza), *OB* O. Basso, *RJ* R. Julián, *RP* R. Pozner, *RS* R. Subils, *SV* S. Vogel. Abbreviations: s.n. without number. An # indicates samples that were measured by flow cytometry

common in western and southern Argentina, reaching the 43.2° of latitude, and the hexaploid (2n = 72, in 32% of the samples) predominates in the central region (within 34.36°–25.11°S and 66.34°–60.48°W) and expands into eastern Argentina (Fig. 2). The Andean areas are colonized mainly by diploids with a few tetraploid populations (always below 1,500 m a.s.l.), while no hexaploids were detected (Fig. 2a).

Table 2 Ploidy level and mean C-values of the different populations of *S. elaeagnifolium* measured

Population (# repeats)	Ploidy level	Mean 2C-value in pg (±SD)	Cx-value in pg	Internal standard
GB 1944 (6)	2x	2.482 (0.012)	1.241	M
NI 1529 (3)	2x	2.496 (0.007)	1.248	M
EF 79 (3)	4x	5.004 (0.009)	1.251	M
LG 239 (3)	4x	5.020 (0.006)	1.255	T
GB 2010 (6)	4x	5.128 (0.030)	1.282	T
AL 17 (9)	6x	7.482 (0.014)	1.247	M
MS 1 (12)	6x	7.38 (0.014)	1.230	M
RJ 39 (6)	6x	7.638 (0.013)	1.273	M

Internal standards: T = *Solanum lycopersicum* cv. Stupické; M = *Zea mays* cv. CE-777. For each population, 1–3 individuals measured, 3 repeats each. Collection data in Table 1

Considering annual precipitation, diploids showed the widest distribution, from localities with 30–926 mm (mean 383 mm). Tetraploids are present in places with 117–737 mm (mean 397 mm) and apparently do not grow in the eastern areas with an annual precipitation higher than 500 mm. Hexaploids inhabit only areas with 567–966 mm (mean 721 mm) (Fig. 2b). At a 0.05 singnificance level, ANOVA tests support these facts: hexaploids inhabit in wetter areas than diploids and tetraploids, although diploids and tetraploids do not differ between them. On the other hand, ploidy levels are not related to variations in annual mean temperature nor altitude.

Nuclear DNA content

The 2C nuclear DNA content of eight accessions of *S. elaeagnifolium* with different ploidy levels was determined (Table 2). Fluorescence histograms (Fig. 3) of relative nuclear DNA content showed distinct G0/G1 peaks with coefficients of variation usually below 4.0%. The 2C DNA content varies threefold from 2.482 to 7.638 pg among the three cytotypes.

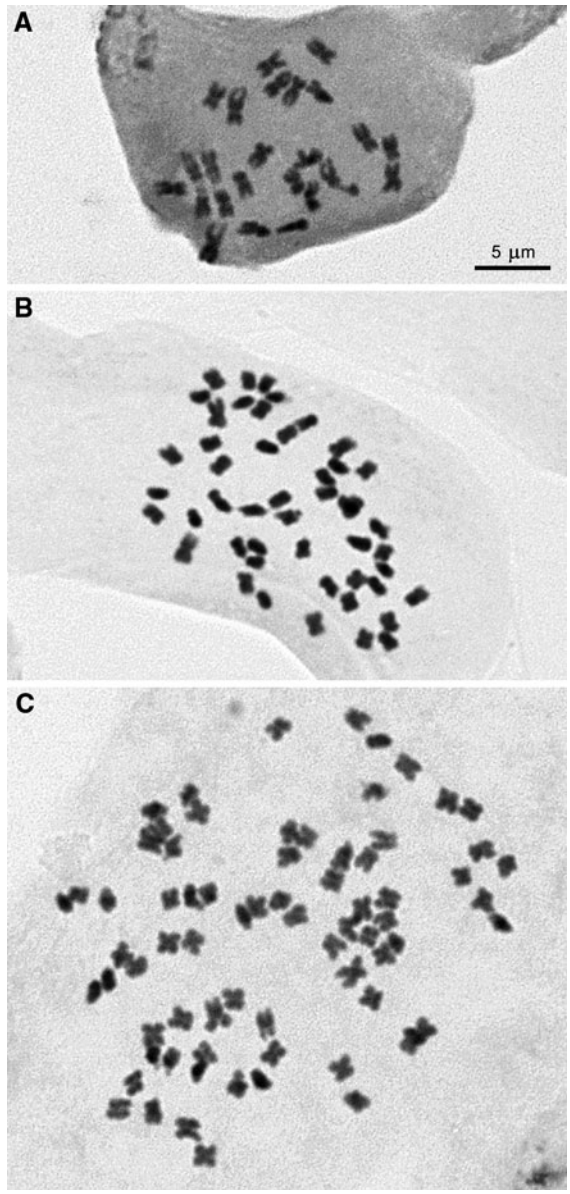

Fig. 1 Photomicrographs of somatic metaphases of *S. elaeagnifolium* accessions with different ploidy levels. **a** Diploid cytotype (2n = 24). **b** Tetraploid cytotype (2n = 48). **c** Hexaploid cytotype (2n = 72). All at the same scale

Cx-values ranged from 1.231 pg for the hexaploid MS 1 to 1.282 pg for the tetraploid GB 2010 (Table 2). Small but significant differences (of about 24.5–50.9 Mbp; 1.02–1.04 fold) were found among accessions, even of the same ploidy level (Table 3). The differences are marginally significant between AL 17 and RJ 39, and between MS 1 and GB 2010.

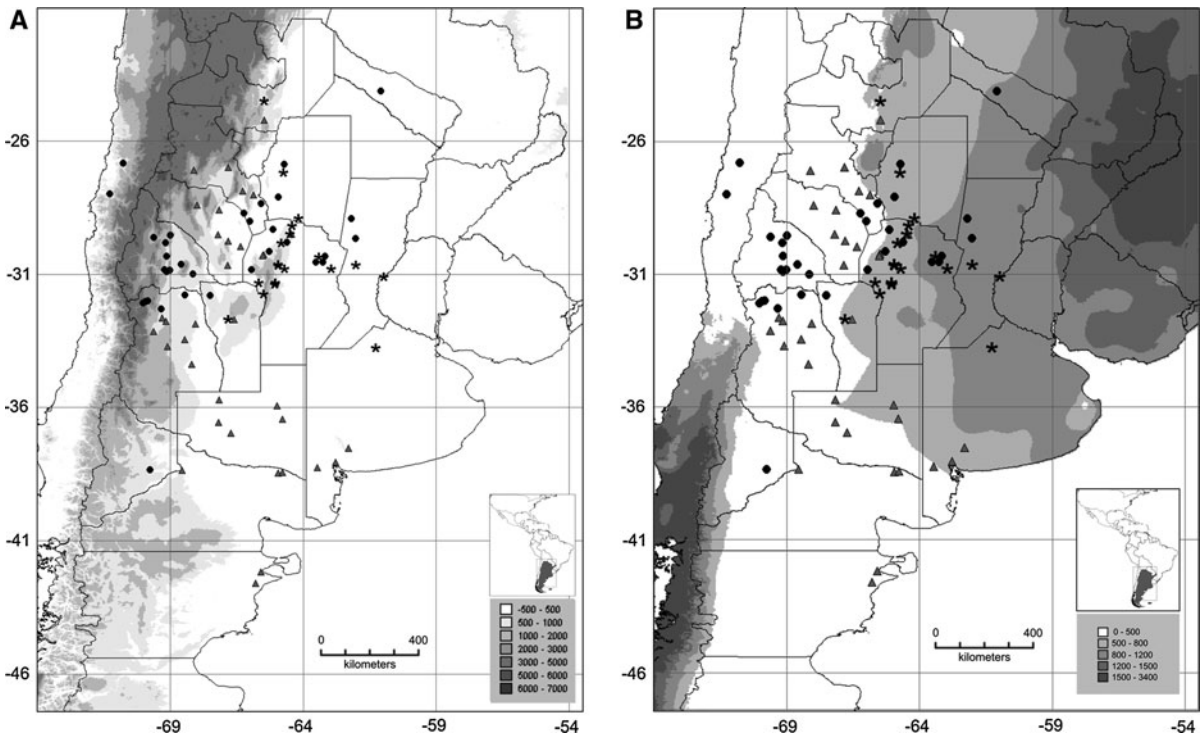

Fig. 2 **a** Elevation map showing the distribution in Argentina of populations of *S. elaeagnifolium* with different ploidy levels. **b** Annual precipitation map showing the distribution of populations of *S. elaeagnifolium* with different ploidy levels. *Circles* Diploids. *Triangles* Tetraploids. *Asterisks* Hexaploids

Principal component analysis (PCA)

A precise separation of the cytotypes with PCA using longitude, latitude, altitude annual precipitation and annual mean temperature cannot be obtained (Fig. 4). Nevertheless, hexaploids generally fall in the upper quadrants, especially the upper right one and the diploids occur in the two right quadrants, while tetraploids are scattered all over the diagram. For Principal Component 1, which explains 55% of the variability, the most important variable was longitude (Eigenvector = 0.55), followed by the precipitation (Eigenvector = 0.52). For Principal Component 2, which explains 28% of the variability, the most important variable was latitude (Eigenvector = 0.79).

Discussion

The importance of polyploidy in angiosperm speciation has been recently re-examined (Soltis et al. 2003; Hegarty and Hiscock 2008; Leitch and Leitch 2008; Van de Peer et al. 2009), and is regarded as a frequent phenomenon. Although most species of *Solanum* subgen. *Leptostemonum* are diploid with 2n = 24 (Chiarini and Bernardello 2006), there are a few cases of polyploid species registered in Argentina: *S. elaeagnifolium*, *S. hieronymi* Kuntze, *S. comptum* C.V. Morton and *S. juvenale* Thell. (Moscone 1992; Chiarini 2008). The polyploid species identified to date are widespread perennial herbs with rhizomes, growing in human-modified environments. Apparently, the areas settled by invasion floras or with unstable environmental conditions, favour the origin and expansion of polyploids, which are better adapted than their diploids relatives to more extreme ecological environments (Lewis 1980). Stebbins (1985) pointed out that polyploidy is more common in species with fragmented distribution, and the secondary contact between diploids and polyploids populations previously isolated would make polyploids more successful, perhaps because of the advantages conferred by polyploidy: gene rearrangements, changes in gene expression, and increased heterozygosis as a

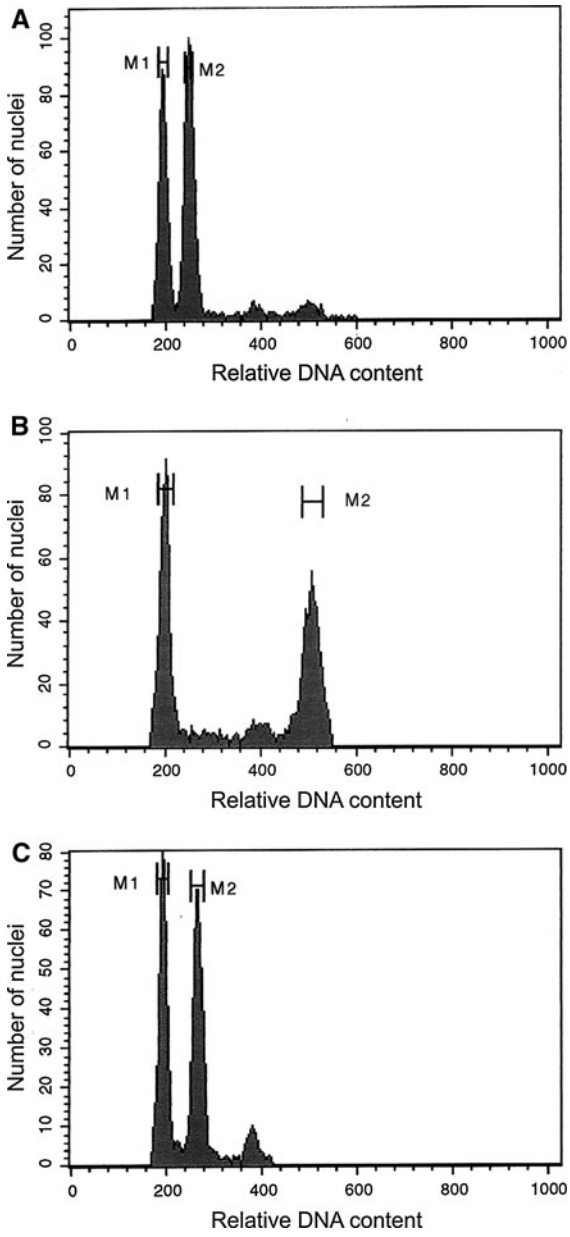

Fig. 3 Fluorescence histograms illustrating the relative nuclear DNA content of the three *S. elaeagnifolium* cytotypes, obtained by flow cytometric analysis of propidium iodide stained nuclei isolated from young lives. **a** Diploid, NI 1529. **b** Tetraploid, GB 2010. **c** Hexaploid, AL 17

result of tetrasomic inheritance allowing the maintenance of 3–4 alleles at a single locus (Stebbins 1985; Soltis et al. 2003), among others.

Conversely, species with weedy features are prone to duplicate their genomes, and polyploidy is referred as common in invasive plants growing far from their native ranges (Ansellem et al. 2001; Lowry and Lester 2006). In this sense, the presence of a polyploid series could suggest that *S. elaeagnifolium* might not have originated in Argentina (probably transported by Spanish settlers), being North America a more plausible place of origin. Clearly, more chromosome counts on North American materials are necessary, because there is only one count (2n = 24) from the USA. In addition, chromosome reports from places where *S. elaeagnifolium* is certainly an invader are lacking.

Regarding the DNA content of weeds, it is worth mentioning that Bennett et al. (1998) compared 116 weeds to 2,473 non-weeds. They reported a range of mean DNA amount per genome between 0.35 and 19.10 pg, with an average of 3.79 pg for the 'weeds', and a range of 0.11–178.95 pg for the 'non weeds', with an average of 12.14 pg. Our values on *S. elaeagnifolium* clearly are within the weed category. In the same study polyploidy was significantly more frequent in weeds (51%), with more polyploids being associated with increasing nuclear DNA amount, and reaching 100% in weeds with the highest 4C DNA amounts. These data reflect an evident relationship between polyploidy, perhaps like that in *S. elaeagnifolium*.

Table 3 Differences in Cx-values among populations of *S. elaeagnifolium*

	AL 17 (6x)	EF 79 (4x)	GB 1944 (2x)	LG 239 (4x)	MS 1 (6x)	NI 1529 (2x)	RJ 39 (6x)
EF 79 (4x)	0.998						
GB 1944 (2x)	0.962	0.788					
LG 239 (4x)	0.841	0.994	0.341				
MS 1 (6x)	0.152	0.796	0.714	*0.019*			
NI 1529 (2x)	1.000	0.999	0.929	0.850	0.196		
RJ 39 (6x)	0.056	0.229	*0.019*	0.254	*0.001*	0.096	
GB 2010 (4x)	0.273	0.389	0.157	0.488	0.066	0.287	0.993

Significant p values (≤ 0.05), according to the Games–Howell test, are italicized. Ploidy levels in brackets. Collection data in Table 1

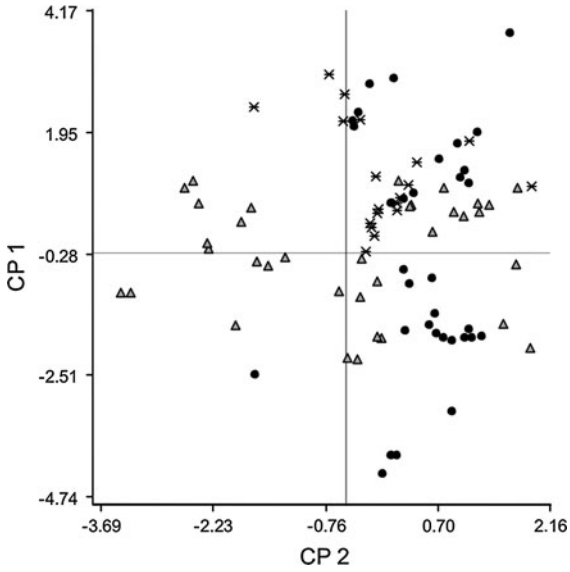

Fig. 4 *Scatter diagram* showing the samples of the three cytotypes in the space defined by Principal Components 1 and 2. *Circles* Diploids. *Triangles* Tetraploids. *Asterisks* Hexaploids

The distribution pattern of *S. elaeagnifolium* in Argentina is probably a response to soil moisture, as rainfall is correlated to longitude and latitude, decreasing from the north to the south and westwards. Western and southern Argentina, where tetraploids live, are arid or semi-arid regions with a mean annual rainfall less than 500 mm (De Aparicio and Difrieri 1958) (Fig. 2b). In contrast, hexaploids inhabit central and eastern areas, where temperatures are milder and mean annual rainfall is 500–1,000 mm (Fig. 2a, b). In the Andean region, diploids and a few tetraploids grow; even though this is a dry area with less than 500 mm, soil characteristics might influence their presence.

A relationship of geographical range and polyploidy has been also shown in other *Solanum*, as the Andean, tuber-bearing section *Petota* (Hijmans et al. 2007). These authors found that polyploids mostly occur in wetter areas near the equator and in drier areas at higher latitudes. In *S. elaeagnifolium*, further ecological studies, including such things as germination tests for moisture stress, would be useful to understand the observed pattern.

The diploid cytotype has a great adaptive capacity, since it exhibits the widest distribution (including wet and dry areas) and is the only one living in salty soils at saline depressions. Nevertheless, diploids are absent from the hills of San Luis and Córdoba provinces (Fig. 2a), where hexaploids are the only cytotype found.

The distribution pattern of *S. elaeagnifolium* cytotypes, with diploids neighboring polyploids, suggests multiple places of origin for its polyploidy in Argentina. At the same time, it is not probable that the hexaploid populations from Salta and Buenos Aires provinces, separated for more than 1,000 km, have a single origin. Additional genetic studies are needed to confirm this hypothesis.

There are examples in which changes in ploidy level have been correlated with morphological and biochemical features (e.g. Masterson 1994; Bureš et al. 2004; Tatum et al. 2006). *Solanum elaeagnifolium* is a highly polymorphic species, and further studies considering morpho-anatomical traits are necessary to establish a possible relationship between polymorphism and ploidy level. Another interesting issue to study is the phytochemistry of its cytotypes, since it is known that polyploidy may increase the production of secondary metabolites (Dhawan and Lavania 1996).

Solanum elaeagnifolium invasiveness can be related to several traits: copious production of sexual and asexual propagules, facility for long distance dispersion, capacity to tolerate considerable drought, and resistance to herbivory, specifically, toxicity to cattle. In addition, irrigation and animal production have facilitated the invasion of semi-arid regions. Its creeping horizontal and deep vertical roots make it very difficult to eradicate once established. Its phenotypic plasticity enables it to adopt different growth patterns to escape mechanical control. The comparison of these traits among the three cytotypes could help to a better understanding of the polyploidy in *S. elaeagnifolium*.

There are several examples of the relationship between DNA content and chromosome size (Nagl and Ehrendorfer 1974; Dimitrova and Greilhuber 2000; Garnatje et al. 2004). In *S. elaeagnifolium*, the Cx-values are in the middle of the known range of *Solanum* subgen. *Leptostemonum* (0.98–2.68 pg; Bennett and Leitch 2010) and these values are consistent with the available karyotype information. In fact, *S. elaeagnifolium* has a mean chromosome length (c) of 1.74 μm and a mean haploid karyotype length (HKL) of 28.83 μm, while *S. atropurpureum* Schrank, a species with a lower DNA content (1C = 1.13, Bennett and Leitch 2010), has smaller chromosomes (c = 1.60;

HKL = 19.12; Acosta et al. 2005), and *S. mammosum* L., with a higher DNA content (1C = 2.68; Bennett and Leitch 2010) has also large chromosomes (c = 2.55; HKL = 28.04; Chiarini and Bernardello 2006).

In some plant genera, DNA content is related to life form, with annuals having lower DNA amounts than perennials (e.g. Albach and Greilhuber 2004; Price et al. 2005). *Solanum elaeagnifolium* has a medium-sized genome compared to other species in the *Leptostemonum* clade (Bennett and Leitch 2010). For example, three annuals (*S. incanum* L., *S. melongena*, and *S. atropurpureum*) have DNA contents lower than *S. elaeagnifolium*, although these few data are not enough to corroborate any relationship. In any case, more measurements in closely related species are needed to study whether there is a relationship of DNA content and life form, as observed in the genera of other families (Albach and Greilhuber 2004).

Understanding the relationships between nuclear DNA content and environmental conditions is complex, because data are contradictory among various studies (e.g. Sims and Price 1985; Palomino and Sousa 2000). However, most support the idea that frequency of species with large 2C-values declines with increasing dryness (i.e., decreasing annual precipitation; Price et al. 1981; Wakamiya et al. 1993; Knight and Ackerly 2002). Considering the 2C-values here obtained for *S. elaeagnifolium* and disregarding the ploidy level, it is evident that the highest values are among samples from the wettest localities, i.e. hexaploids in eastern areas. In fact, Knight and Ackerly (2002) point out that frequency of species with large 2C-values declines with decreasing annual precipitation, while species with small 2C-values predominate in all environments.

Genome size variations have been also associated with geography, habit or altitude (e.g. Bureš et al. 2004; Šmarda and Bureš 2006). In *Lagenaria siceraria* (Molina) Standl. (Cucurbitaceae), for instance, Achigan-Dako et al. (2008) found that the higher the land elevation, the larger the DNA content of the samples, and this was also correlated to morphological traits. Šmarda and Bureš (2006) found that three tetraploid populations of *Festuca pallens* Host (Poaceae) have a relative DNA content significantly smaller than twice of the diploids, but at the same time the content of the tetraploids varied among them, according to geographical distribution. Nevertheless, in *S. elaeagnifolium* samples studied here, variations in Cx-values cannot be attributed to a cause other than intraspecific variability, since altitude and temperature data are not significantly different among cytotypes.

As in *S. elaeagnifolium*, small genome size variations have been found in genera of other families. For example, Vaio et al. (2007) found 1.016–1.066 fold (1.6–6.6%) differences among Cx-values of *Paspalum dilatatum* Chirú (Poaceae) polyploids with the same genome formula. Accessions of the diploid *Arachis duranensis* Krapov. et W.C. Gregory (Leguminosae) differed up to 1.065 fold (6.5%, Temsch and Greilhuber 2001). The small variations found here suggest a relative stability in *S. elaeagnifolium* genome size, compared to other species with high variations (e.g. *Sorghum*, Price et al. 2005; *Sinningia*, Zaitlin and Pierce 2010). A lack of genome-size variation has been found in several cultivated species, such as common pea, soybean, and pigeonpea (Greilhuber and Ebert 1994; Greilhuber and Obermayer 1998). It seems possible that intraspecific variation in genome size depends on isolation of populations: with more isolation, more variation. The differences in DNA content among populations with the same ploidy level can be attributed to long-term isolation, and it is more probable to be detected in wild species (like *S. elaeagnifolium*) than in cultivated ones.

Genome downsizing is another discussed phenomenon that apparently occurs in polyploids (Leitch and Bennett 2004). Polyploids are expected to have larger C-values than their diploid progenitors, increasing in direct proportion with ploidy. This is true in some polyploid series, especially those newly formed, but Leitch and Bennett (2004) provided examples of particular polyploids in which C-values did not follow such a rule. In fact, these authors found two different cases: either the mean 1C DNA amount did not increase proportionally with ploidy, or the mean DNA amount per basic genome (2C value divided by ploidy) tended to decrease with increasing ploidy. In the accessions here studied, we found significant or marginally significant differences in Cx-values which are examples of the two types of deviation. Thus, one of the hexaploids (MS 1) has a Cx-value smaller than some tetraploids (GB 2010 and LG 239), but one hexaploid (RJ 39) has a greater value than one of the diploids (GB 1944). In addition, there are differences in DNA content among the hexaploids examined (MS 1 and AL 17 have lower values than the value of RJ 39). All these facts suggest two possibilities: either some

polyploid populations would have originated recently and no genome downsizing would have occurred yet, or there would be several places of origin for polyploids in Argentina. A study considering DNA measurements in samples from countries other than Argentina could help answer these questions.

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